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Impact of hemicellulose side chains modifications on plant cell wall mechanical properties

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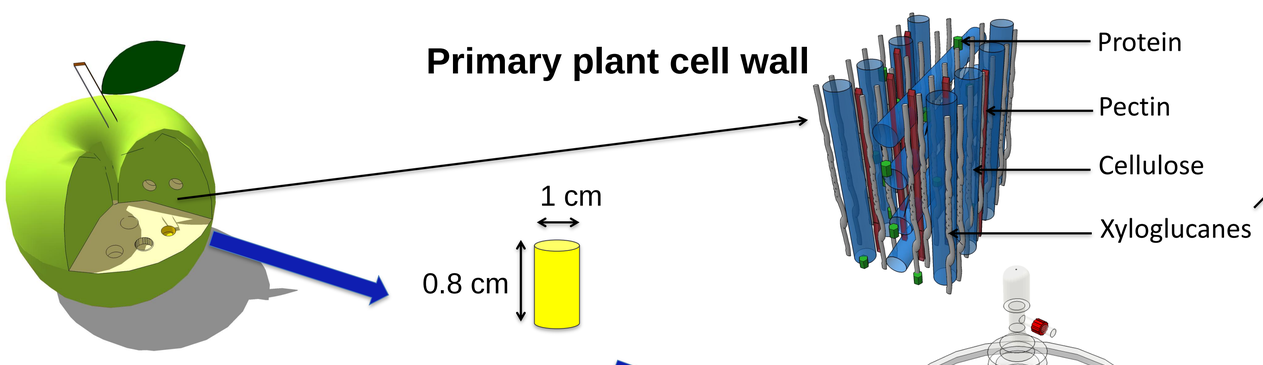
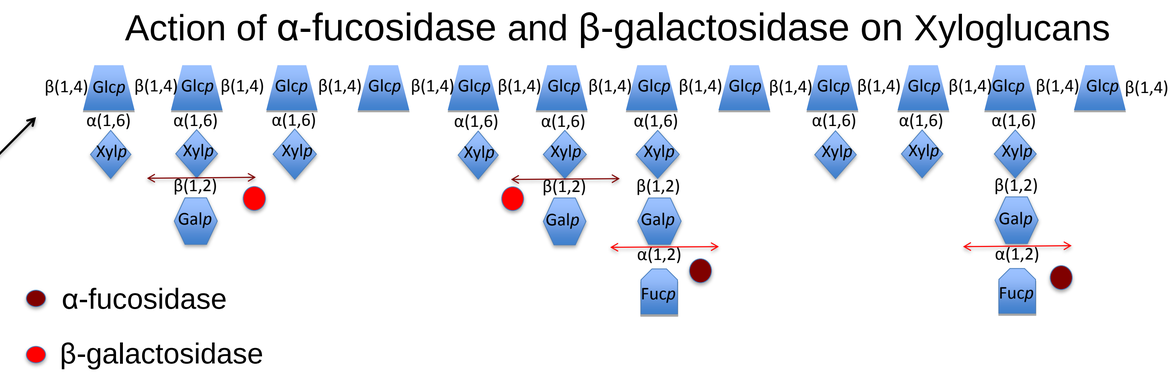
Plant development with erected stature and exploration of the environment relies on tissue mechanical properties. These are determined by the turgor pressure, spatial organisation of cells in tissue and cell wall chemistry and organisation. The growing dicot plant cell wall is mostly composed by polysaccharides (cellulose, pectin, hemicellulose) and small amounts of proteins and lignin. During development, the polysaccharide network is build, reassembled and disassembled by numerous enzymes leading to significant changes of mechanical properties.

The aim of this work is to establish relationships between cell wall polysaccharides structure and parenchymatous tissue mechanical properties.

The approach used consists in a targeted alteration of the fine structure of cell wall polysaccharide by specific enzymes combined with the monitoring of the induced changes in mechanical properties at the tissue scale. The targeted polysaccharide structural domains, are xyloglucan side chains. In spite of the major role of this hemicellulose on the cell wall load-bearing network, the function of its side chains on mechanical properties has not been reported.

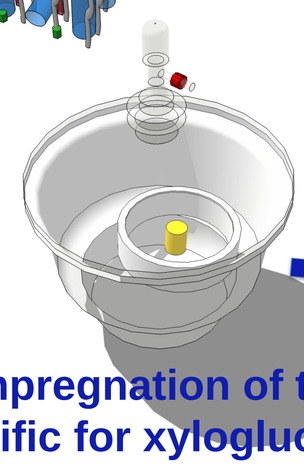
This study is performed on parenchymatous tissues from two contrasted apple varieties Golden delicious and Granny Smith. These varieties differ in their polysaccharide composition and texture but share close histological structure.





1- Sampling

Parenchyma tissue are sampled as cylinders with a cork-borer following radial and longitudinal axes in order to consider apple parenchyma anisotropy. 4 apples are used per condition, for each apple, 5 cylinders are controls and 4 are tests.



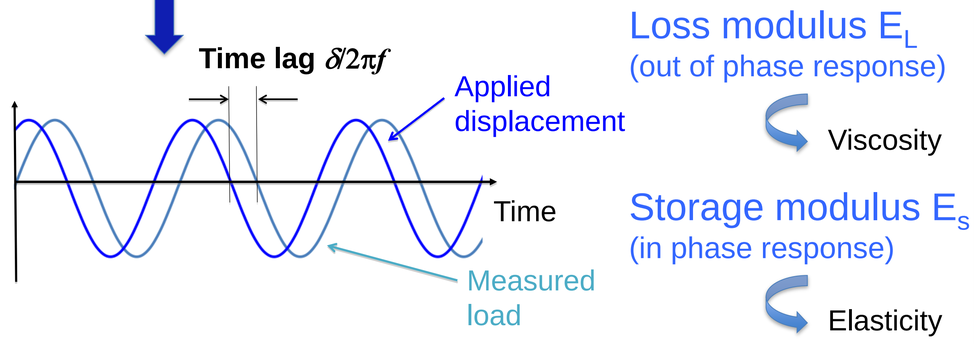
2- Vacuum impregnation of tissues with enzyme specific for xyloglucan side chains

In order to preserve turgor pressure of parenchyma samples, enzymes are infused in an isotonic buffer (mannitol, ascorbic acid, MES, Ca^{2+} and DMSO, pH 6). This buffer was designed to maintain mechanical properties of samples for at least 5 hours without added enzymes. Vacuum infusion is realized by maintaining samples in buffer ± enzymes at 50 mbar during 30 s followed by a slow vacuum release.

3- Mechanical assay on apple parenchyma infused by buffer with or without enzyme

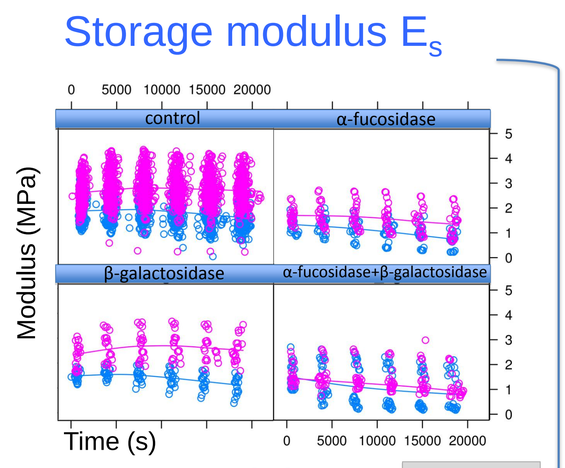
Determination of loss and storage moduli at a frequency f of 1 Hz during 5 hours (BOSE®-Electroforce 3230 DMA)

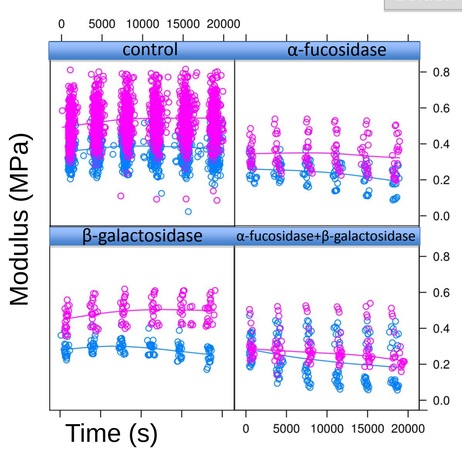
4- Data processing



5- Data analysis

Consequence of enzymatic degradation of xyloglucan side chains on the mechanical behaviour





Polynomial regression

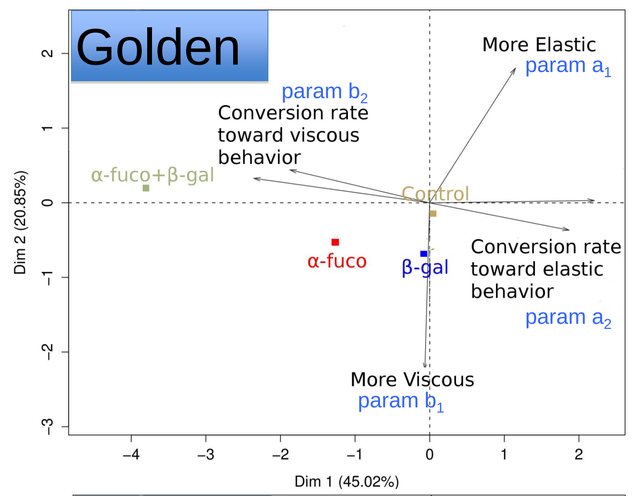
$E_s = a_1 t^2 + a_2 t + a_3$

$E_l = b_1 t^2 + b_2 t + b_3$

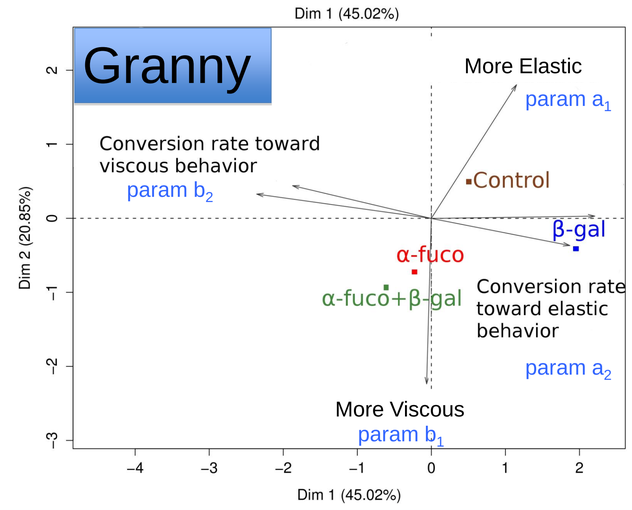
Parameters

$a_1, a_2, a_3, b_1, b_2, b_3$

Principal component analysis



- β-galactosidase increases viscous behavior
- α-fucosidase increases viscous behavior
- β-galactosidase + α-fucosidase increase viscous behaviour and increase the conversion rate toward viscous behavior



- β-galactosidase increases viscous behavior
- α-fucosidase increases viscous behavior
- β-galactosidase + α-fucosidase increase viscous behavior

Alteration of xyloglucan side chains causes important modifications of mechanical properties at the macroscopic scale. These results emphasize the key roles of side chains in the load-bearing implication of xyloglucan in cell wall mechanical characteristics either directly in changing polysaccharides interactions or indirectly in regulating endogenous enzymes activity. These contrasted effects in Golden and Granny apple illustrate the diversity and complexity of cell wall with regards to their role on fruit development and maturation.

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